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Reduction in peroxide values and monocarbonyls of oxidized methyl oleate by several microbial cultures

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With oxidized methyl oleate as the substrate, a rapid method for the determination of microbial destruction of peroxides and monocarbonyls has been developed. *Staphylococcus aureus* showed extensive destruction of peroxides by both cells and supernatant fluid, while *Bacillus cereus*, *Micrococcus cryophilus*, *Serratia marcescens*, and *Sarcina lutea* had activity only in the cells. *Escherichia coli* was inactive. Destruction of 2,4-dienals, 2-enals, and *n*-alkanals followed a similar pattern except that *E. coli* cells also were active against the monocarbonyl classes. Paper chromatography did not reveal any selective action against specific monocarbonyls within the classes.

Introduction

Recent studies from this laboratory (12) reported that peroxides and carbonyls found in rancid lard often were decreased by the action of microorganisms. Although investigations on the importance of this activity in foods ultimately must be made on naturally occurring fresh and oxidized fats, the relatively long periods of incubation required (3–5 days) for these substrates are inconvenient for the screening of cultures for possible activity. This report is concerned with the development of a more rapid method of assay and its use to investigate the action of selected microorganisms on the peroxides and monocarbonyls of oxidized methyl oleate.

Methods

Microorganisms—The cultures used in this investigation were from our stock culture collection. Portions of 18- to 24-h aerated cultures grown in the broths indicated in Table I were frozen and used to prepare the seed cultures with which the final growth medium was inoculated. Whole cultures, supernatant fluid from these cultures, and cells washed twice with 0.018 *M* disodium phosphate buffer and resuspended in buffer were assayed for activity. Portions of these samples, autoclaved at 121 C for 15 min, served as controls.

Preparation of oxidized methyl oleate—Methyl oleate (Eastman Organic Chemicals) was spread in a thin layer and oxidized by exposure to ultraviolet irradiation at room temperature until a peroxide value (PV) of 50 to 250 was reached.

Determination of peroxides—The PV was determined by the cold iodometric method of Lea (5). Centrifugation was used to facilitate breaking the emulsion during extraction with petroleum ether.

Determination of monocarbonyls—A portion of the solvent fraction obtained above, containing about 1 g of methyl oleate, was reacted with 2,4-dinitrophenylhydrazine on the Schwartz column (9). The monocarbonyls were recovered by column chromatography as described by Schwartz and associates (9, 10) and separated into classes by the method of Gaddis and Ellis (3). Classes were separated into individual compounds and the compounds C_6 and above were estimated by paper chromatography as described by Ellis *et al.* (1). Any compounds below C_6 were concentrated in the C_5 position by this method, but thin-layer chromatography (7) showed that compounds below C_5 seldom were present.

Results

Development of Assay

Since *Staphylococcus aureus* No. 63 had definite peroxide-decomposing activity, it was selected as the test organism for development of the assay. Emulsions containing lard as the substrate were unstable when shaken during incubation; therefore, the methyl ester of oleic acid, the major fatty acid in lard and one on which numerous oxidative studies have been made (2, 4, 8, 11, 13), was selected as the substrate. One milliliter of methyl oleate in 30 ml of culture formed a stable emulsion and this ratio was selected for the assay since these were convenient volumes to handle. Higher concentrations of substrate did not increase the rate of decomposition, whereas increasing the volume of culture did increase activity. These data indicated that the methyl oleate was in excess for the assay system selected.

Agitation of mixtures of oxidized methyl oleate and either sterile broth or autoclaved

cultures for at least 12 h at 400 cycles/min on a reciprocating waterbath shaker (2-in. stroke) caused no change or a slight (5–10%) decrease in the initial PV. Thus, there was no added oxidative effect on the substrate caused by the aeration of the mixture during shaking. When aeration speeds were compared, it was found that reduction in PV was greater at 200 cycles/min than at 100 cycles/min, but higher speeds had no increased effect. Figure 1 shows that most of the reduction in PV occurred within the first 20 min of incubation. Therefore, an incubation period of 15 min was selected for subsequent development of an assay for *S. aureus* activity.

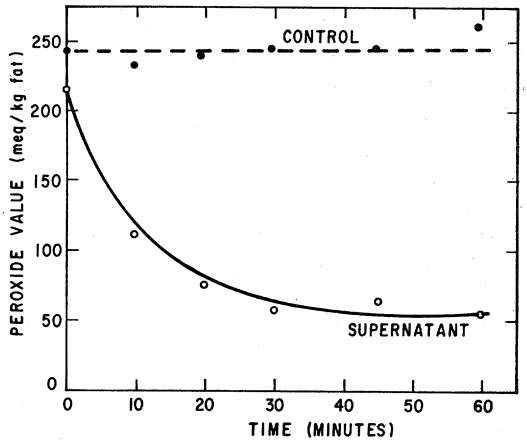

FIG. 1. Activity of the supernatant fluid of a *Staphylococcus aureus* culture in decreasing the peroxide value of oxidized methyl oleate.

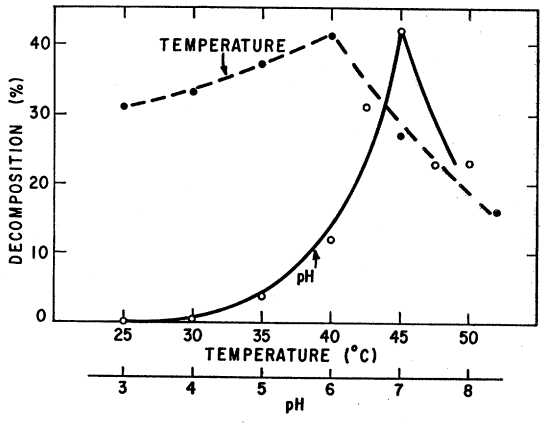

FIG. 2. Effect of pH and incubation temperature on peroxide decomposing activity of *Staphylococcus aureus*.

Figure 2 shows that the optimum pH for the decomposing activity of *S. aureus* was about 7.0 and the optimum temperature was near 40 C.

Final Method of Assay

Oxidized methyl oleate (PV 50 to 200) was added to supernatant fluids adjusted to pH 7.0, or washed cells suspended in 0.018 *M* phosphate buffer, pH 7.0, to give a final concentration of 3.3% (v/v). The mixture was incubated on a reciprocating shaker (200 c.p.m., 2-in. stroke) at 37 C. Because of low activity of several microorganisms, all samples were incubated 2 h instead of the 15 min found to be ample for *S. aureus*. Peroxide values, total monocarbonyls, monocarbonyl classes, and individual compounds were determined on petroleum ether extracts of these samples.

Peroxide Decomposing Activity of Cells and Supernatant

On the basis of decomposition of peroxides by whole cells and supernatant liquid, the cultures fell into three groups (Table I). Group 1, containing the two staphylococci, had extensive activity in both the cells and the supernatant fluids. In the second group, only the cell fraction of *Bacillus cereus*, *Serratia marcescens*, *Sarcina lutea*, and *Micrococcus cryophilus* showed activity when the initial PV was high. *B. cereus*, however, had slight activity in the supernatant when the initial PV was low; this occurred consistently when the initial value was 50 or below. *S. lutea* was not examined at low PV. Neither cells, supernatant fluid, nor whole cultures of *Escherichia coli* showed any activity on oxidized methyl oleate under these conditions regardless of the initial PV. However, as had been shown previously (12), the culture did have activity against oxidized lard.

Microbial Action on Monocarbonyl Classes

Four microorganisms representing the three groups were examined for their action on the three classes of monocarbonyls in oxidized methyl oleate. Table II shows that the supernatant fluids of the organisms were variable in their effect on the various monocarbonyl classes, with three of the four cultures causing an increase in one or more of the classes. The washed cells, however, with one exception, decomposed them. *S. aureus* cells caused an increase in the *n*-alkanal

class of about 35% while those from other organisms decreased this class at least 35%.

Action on Specific Compounds

Within the 2,4-dienals, the C_9 and C_{10} compounds were the major ones present in the oxidized methyl oleate. The C_9, C_{10}, and C_{11} chain lengths were predominant in the 2-enals and C_9 and C_{10} in the alkanals. In addition, small amounts of many of the compounds within the C_7–C_{12} range were present. In none of the samples, however, could the decrease in monocarbonyls be related to more than specificity for the class of compounds. For example,

TABLE I

Decomposition of peroxides by cells and supernatant fluids of several microorganisms

Group	Microorganisms	Initial PV	% decomposition	
			Supernatant	Cells
No. 1	*S. aureus* No. 63[a]	201	76	37
		107	27	33
	S. aureus No. 66[b]	87	56	35
No. 2	*B. cereus* No. 284[c]	48	12	14
		204	0	38
		80	0	26
	M. cryophilus No. 90[d]	90	5	33
		55	0	40
	S. marcescens No. 279[d]	92	0	21
		206	0	16
	S. lutea No. 112[c]	94	0	28
No. 3	*E. coli* No. 107[c]	90	0	0
		59	0	0
		50	0	

[a]Grown in Difco Tryptose Phosphate Broth + 2% tryptose at 37 C on shaker (190 r.p.m., 2-in. stroke) for 18–24 h.
[b]Same as [a] except no additional tryptose added.
[c]Grown in Difco Veal Infusion Broth, 25 C, shaker, for 18–24 h.
[d]Same as [c] except 20 C.

TABLE II

Decomposition of monocarbonyls by cells and supernatant fluids of four bacteria

	PV[a]		2,4-Dienals		2-Enals		*n*-Alkanals	
	Control	Sample	Control, μmol/10 g fat	% decomp.	Control, μmol/10 g fat	% decomp.	Control, μmol/10 g fat	% decomp.
S. aureus								
Cells	106	71	1.7	47	9.9	36	30.6	[b]
Supernatant	107	80	1.5	[b]	11.3	9	33.7	[c]
M. cryophilus								
Cells	59	35	1.5	75	8.6	64	24.6	60
Supernatant	51	52	1.3	0	6.8	4	20.2	7
B. cereus								
Cells	50	43	0.79	94	5.6	75	18.0	44
Supernatant	49	44	0.97	18	5.8	[c]	16.6	[c]
E. coli								
Cells	58	59	1.2	100	10.6	25	30.6	35
Supernatant	62	66	0.82	51	7.1	[c]	24.7	[c]

within the 2-enals, cells of all four bacteria decomposed some part of this group, but the paper chromatograms did not show a consistent effect on any one of the specific compounds.

Discussion

The similarity in action of all cultures studied except *E. coli*, coupled with the rapidity of the assay, indicates that the use of methyl oleate rather than a more complex substrate, such as lard, has value in a screening procedure to isolate cultures able to attack monocarbonyls. The variable patterns of activity among different microorganisms and the evidence for an optimum pH and temperature suggest that this activity, if not enzymatic, is closely related to some enzyme system.

The inability of *E. coli* to attack the peroxides of methyl oleate, while active against the peroxides of lard, suggests that the peroxides in methyl oleate were either refractory to the enzyme system of *E. coli* or inhibitory to it. The first possibility is less likely since oleic acid is a major component of lard and they should contain many peroxides in common when oxidized. That possibility also implies a high degree of substrate specificity not found among lipolytic enzymes like lipase or in the data reported here on compounds within the monocarbonyl classes.

It has been reported (6) that the rate of thermal decomposition of hydroperoxides in methyl oleate is directly related to their initial concentration. In the present study, the supernatant from *S. aureus* and washed cells of *B. cereus* also exhibited a higher peroxide decomposing activity when the initial concentration was high. The increased activity in these instances with a high initial PV could be explained by accumulation of breakdown products which in turn catalyze the reaction as occurs in thermal decomposition. On the other hand, the lack of activity when the initial PV was high, as was exhibited by the supernatant fluid from a *B. cereus* culture, could be caused by a specific inhibitory peroxide interfering with the initiation of enzymatic activity.

When percentage decomposition alone is considered, the 2,4-dienals are more susceptible to bacterial attack than the other monocarbonyls. However, this may be a concentration effect because relatively small amounts of the 2,4-dienals are present originally. Until a procedure is developed for the more rapid extraction, quantitation, and identification of individual monocarbonyls, chemical analyses beyond the classes of monocarbonyls is of doubtful value.

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